TITLE: Antioxidant capacity of cookies with non-modified and modified sugar beet fibers: chemometric and statistical analysis

AUTHORS: Dragana Šoronja-Simović, Nikola Maravić, Zita Šereš, Aleksandra Mišan, Biljana Pajin, Lidija Jevrić, Sanja Podunavac-Kuzmanović, Strahinja Kovačević

NOTICE: This is the author's version of a work that was accepted for publication *European Food Research and Technology*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *European Food Research and Technology*, Volume 243, Issue 2, February 2017, Pages 239–246. DOI: 10.1007/s00217-016-2739-4

Antioxidant capacity of cookies with non-modified and modified sugar beet fibres: chemometric and statistical analysis

Journal:	*European Food Research and Technology*
Manuscript ID	EFRT-15-1460
Manuscript Type:	Original paper
Date Submitted by the Author:	21-Oct-2015
Complete List of Authors:	Maravić, Nikola; University of Novi Sad, Faculty of Technology Šoronja Simović, Dragana ; University of Novi Sad Šereš, Zita ; University of Novi Sad Mišan, Aleksandra; Institute of Food Technology Pajin, Biljana; University of Novi Sad Jevrić, Lidija ; University of Novi Sad Podunavac-Kuzmanović, Sanja; University of Novi Sad Kovačević, Strahinja; University of Novi Sad
Keywords:	dietary fibre, modification, antioxidant activity, DPPH, chemometric analysis, sum of ranking differences

Antioxidant capacity of cookies with non-modified and modified sugar beet fibres: chemometric and statistical analysis

Nikola Maravić [a,1], Dragana Šoronja Simović[a], Zita Šereš[a], Aleksandra Mišan[b], Biljana Pajin[a], Lidija R. Jevrić[a], Sanja O. Podunavac-Kuzmanović[a], Strahinja Z. Kovačević[a]

[a] *University of Novi Sad, Faculty of Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia*

[b] *Institute of Food Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia*

Abstract

Recent studies have confirmed the possibility of an insoluble material to carry out a marked antioxidant activity by a solid-liquid interaction and in such way opened a new chapter for dietary fibre application in food industry as a functional unit with radical scavenging capacity. Therefore, this paper investigates the possibility of improving the antioxidant activity of cookies with addition of sugar beet fibres. The chemometric analysis was carried out on the experimentally obtained data of the antioxidant activity of the cookies with modified and non-modified sugar beet fibres compared to the cookies with commercially available dietary fibre (Fibrex®) produced in the Nordic Sugar A/S factory, Sweden. The hierarchical cluster analysis and sum of ranking differences were applied. The introduction of modified and non-modified sugar beet fibres in the cookies formulation showed promising results regarding the cookies EC_{50} values decline compared to the control samples. Cookies

[1] Corresponding author. Address: Bul. cara Lazara 1, 21000 Novi Sad, Serbia; Tel.: +381 21 485 3685; E-mail address: maravic@tf.uns.ac.rs (N. Maravić)

with addition of modified sugar beet fibres showed best antioxidant activity in the first 4 weeks. Cookies with Fibrex® fibres exhibited highest antioxidant activity.

Keywords: dietary fibre; modification; antioxidant activity; DPPH; chemometric analysis; sum of ranking differences.

Introduction

The requirements of the recommended daily intake of the dietary fibre encouraged the wide research focusing on their extensive application in human nutrition [1]. Insufficient dietary fibre intake has been associated with a variety of diseases such as obesity, constipation, appendicitis, type 2 diabetes, diverticular disease, different cardiovascular diseases and bowl cancer [2]. Therefore, a wide range of fibre sources are developed in order to provide functional food with sufficient amount of fibre content [3], [4].

The American Association of Cereals Chemists Expert Committee defined dietary fibre as an insoluble material formed by a cell wall polysaccharides, lignin and associated substances resistant to hydrolysis by the digestive enzymes of humans [5]. The recent finding regarding the possibility of an insoluble material to exert a marked antioxidant activity by a solid-liquid interaction opened a new chapter in the physiological relevance of the food insoluble material [6].

A variety of fibres from the plant sources have been used in a food products to enhance the structure, color and aroma with a reduced energy of the final products [7]. Recently, sugar beet fibre has been introduced in the food technology as a fibre source [8]. The chemico-nutritional composition of the sugar beet pulp as a by-product of a sugar

refining industry suggests the possibility of use of sugar beet pulp as raw material for dietary fibre production [9].

Previous investigations of the effect of sugar beet fibre addition on cookies quality showed promising results regarding the particle size, colour and odour properties of the cookies [10]. *Gyura et al.* [11] determined the physicochemical characteristics of sugar beet fibres both untreated and treated with peroxide or sulphite ions. According to the results of their research insoluble fibre content was in the range of 60-70% and antioxidant activity was based on total phenolic content in the range of 272.94 GAE/g for non-treated fibres and 71.07 GAE/g for the fibres treated with peroxide or sulphite ions.

Ferulic, gentisic and *p*-coumaric acid have been identified and reported to be predominant phenolic acids in the ethanolic extract of sugar beet pulp [12] and have proved to be relatively potent antioxidants [13]. However, the results obtained by *Hęś et al.*[14] suggest that processing conditions must be optimized to retain the health promoting compounds in products in order to avoid oxidation of antioxidant, complexation with other food compounds, enzymatic modification and transition from antioxidant to pro-oxidant.

Therefore, this research will investigate the possibility of introduction of modified (MF) and non-modified (NMF) sugar beet fibres in the formulations of cookies to improve their antioxidant activity and functional characteristics. The chemometric analysis was carried out on the experimentally obtained data of the antioxidant activity of the cookies with modified and non-modified sugar beet fibres compared to the cookies with commercially available dietary fibre (Fibrex®) produced in the Nordic Sugar A/S factory, Sweden.

Materials and methods

The physicochemical properties of sugar beet pulp regarding protein, insoluble and soluble fibre content were determined by *AOAC Official methods* [15]:

Determination of dry matter and minerals was carried out according to the specified methods by *Reinefeld and Schneider* [16]. Non-modified sugar beet dietary fibres (particle size <150μm) were produced under the laboratory conditions. Sugar beet pulp was extracted with sulphurous acid at 75 ^{o}C and pH 5.7 during 60 minutes. Extracted sugar beet pulp was pressed to remove the excess water, dried at 80 ^{o}C, grounded in a laboratory mill (type LM 3100, Falling Number, Perten Instruments, Sweden), and sieved through a laboratory sieve (type SZ-1, ZBPP, Bydgoszoz, Poland) to obtain a fraction with the particle size less than 150 μm which was used for further analyses.

Modified sugar beet fibres were obtained after the treatment with hydrogen peroxide solution up to blend concentration of 20 g/L H_2O_2 at pH 11 adjusted with 10 M NaOH. Neutralisation was conducted using ccHCl. Chlorine ions were removed using distilled water until the negative reaction to Cl^- ions. Afterwards, excess water was pressed out and modified fibres were dried at 80 ^{o}C. This modification was applied in the aim of improving hydration characteristics of fiber and its sensory properties for bakery products [17], [18]. Grounding and sieving of dried modified fibres was conducted using the same procedure as it is described above for NMF.

Control samples of the cookies without sugar beet fibre were produced according to the formulation presented in Table 1. For the preparation of cookies with MF, NMF and Fibrex® fibres 7%, 9% and 11% of wheat flour (T-500) was replaced with the corresponding fibre source. A low speed laboratory mixer (60min^{-1}) was used for preparation of wheat flour, powdered sugar and vegetable fat mixture. The mixture was kneaded after the addition of other components (previously dissolved in water) for 15min. The formed dough rested at 20 ^{o}C and manually shaped in the appropriate form. The circular form cookies were baked in an

air oven at 230 ± 2 ºC for 15 min. Prior to analysis samples were cooled down to room temperature.

(Please insert Table 1 here)

Sample cookies containing 0, 7, 9 and 11% of sugar beet dietary fibres are pulverized and the corresponding sample mass (10 g) was added in a vial. In each vial 40 ml of methanol was added and the extraction is carried out at room temperature for 2 min with vigorous agitation (60 o/min). Filter paper (Whatman, Grade 4 Chr, UK) was used for the separation of the extract. The separation procedure was repeated with 100 mL of solvent two times and extraction solutions were combined and dried in vacuum-evaporator. The dried extract was resolved in 96% ethanol to obtain 10 mL volume. The extract obtained by this procedure was used for further investigation of antioxidant activity.

The antioxidant activity of cookies with sugar beet fibres is determined using the DPPH radical scavenging method. *Hatano et al*. [19] method was used to estimate the content of 1,1-diphenyl-2-picrylhydrazyl (DPPH·) radicals in the examined extracts. The concentration of the DPPH solution which was used in the assay was 90 μM (22.5 mL 0.4 mmol/L DPPH solution (0.01577 g DPPH· in 100 mL methanol) was diluted with 95% methanol to 100 mL). The examined extracts of different concentrations (10, 15, 20 and 25 mg/mL) were added into the DPPH solution diluted in methanol. The DPPH solution (90 μM) was prepared by diluting 1.0 mL of the DPPH· solution (90 μM) in 2.9 mL methanol. The change in the absorption after 60 minutes at 517nm was measured using Jenway, 6405 UV/V and compared to the absorption of the blank sample (without extract). EC_{50} value (mg/mL) was defined as the concentration of an antioxidant extract which was required to

quench 50% of the initial DPPH· under the experimental conditions given. It was obtained by interpolation from linear regression analysis. BHT and α-tocopherol were used as control.

In the present paper, hierarchical cluster analysis (HCA) and sum of ranking differences (SRD) were applied. The purpose of these chemometric methods was to detect similarities or dissimilarities among the analysed samples based on their antioxidant activity (EC_{50}) measured during six weeks.

HCA is a simple method used for dividing a large group of object into smaller groups (clusters) so that similar objects are placed in the same cluster. It searches for objects which are closest to each other in the space of the analysed variables. The clusters are not known before the mathematical analysis and no assumptions are made about the distribution of the variables [20]. The distance between two objects is usually defined as Euclidean distance (*d*):

$$d = ((x_1 - y_1) + (x_2 - y_2) + \cdots + (x_n - y_n))^{\frac{1}{2}} \quad (1)$$

where x_n and y_n are the coordinates of the points in *n*-dimensional space. There are many methods for cluster formation. The most used are Ward's and single linkage methods [21]. The graphical result of HCA is called a dendrogram. It presents the successive stages of clustering of objects. Degree of similarity or dissimilarity among the objects can be determined on the basis of vertical axis of dendrogram. HCA is widely applied method for data analysis in microbiology, analytical chemistry, food engineering [22], etc.

SRD is a relatively new chemometric method for ranking of objects (mathematical models, samples, compounds, etc.) with regard to reference (ideal) ranking or so-called "golden standard". Row average, minimum or maximum row values or experimental values can be set as a reference ranking, depending on the purpose of the analysis. The closer is the SRD value of a model to zero, the better is the model (the ideal model has SRD = 0, since in

that case it has the same ranking as the "golden standard"). SRD corresponds to the principle of parsimony and it is an easy tool for evaluation of the models, methods or samples [23]. If two or more samples have similar SRD values, they are similar according to the analysed variables (close proximity means close similarity). Therefore, the SRD method can be applied for groupings of samples or it can be considered as dissimilarity measure. The SRD analysis is validated by comparison of ranks by random numbers (CRRN procedure), which is a kind of simulation test. CRRN procedure requires the determination of the theoretical distribution function of SRD values corresponding to the number of n objects consisted of random numbers. Recursive algorithm is applied for calculation of theoretical distribution function if $n < 14$, while the normal distribution is used to approximate the theoretical (random) SRD distribution function for large number of objects ($n > 13$) [24].

HCA was carried out by NCSS 2007 and Statistica v.10.0 software. SRD analysis was conducted by using Microsoft Excel 2013 program.

Results and discussion

The physicochemical properties of sugar beet fibres used in cookies preparation are presented in Table 2. Previous studies have shown that physicochemical properties of dietary fibres depend on both processing steps and raw material properties [25]. Results indicate that the ratio of soluble/insoluble fibre is increasing after the modification of sugar beet fibres since the applied treatment of sugar beet fibres resulted in conversion of insoluble protopectin to water soluble pectin and insoluble cellulose and hemicellulose [26].

(Please insert Table 2 here)

The influence of presented physicochemical changes of sugar beet fibres on the cookies' antioxidant activity is evaluated using chemometric analysis of measured EC_{50} values.

Statistical and chemometric analysis

The complete chemometric analysis was based on the data matrix shown in Table 3. This table shows the EC_{50} values, measured during six weeks, of the samples with different quantities of non-modified fibres (7%, 9% and 11%), Fibrex® fibres (7%, 9% and 11%) and modified fibres (7%, 9% and 11%).

(Please insert Table 3 here)

In the first step of the chemometric analysis, the box-whisker plot (Fig. 1) was formed on the basis of the data given in Table 3. As it can be seen from the presented plot, some similarities among the analysed samples can be observed, for example similarity among the control samples including MF-7% sample. Also, the similarities among F and NMF samples can be assumed. However, these assumptions are made just on the simple visual comparison of the ranges of EC_{50} values of the samples, and the application of reliable chemometric (statistical) approach is needed. In this study, this refers to HCA and SRD methods.

(Please insert Fig. 1 here)

Fig. 1 Box-whisker plot of EC_{50} values of the analysed samples

The applied HCA, based on Ward's clustering method and Euclidean distances, resulted in two main clusters, as it can be noticed on the dendrogram in Fig. 2. The cluster 2 contains only the control samples, indicating significant differences between control and other samples.

(Please insert Fig. 2 here)

Fig. 2 The dendrogram as a result of HCA based on Ward's clustering method and Euclidean distances

The samples in cluster 1 are grouped so the samples with Fibrex® fibres are significantly separated from the others. However, regarding the samples with non-modified and modified fibres, it can be seen that the sample with 11% modified fibres (MF-11%) belongs to the cluster which contains the samples with non-modified fibres. This indicates their similarity regarding the EC_{50} values. Their similarity can be mostly attributed to the amount of fibre in the sample (11%) which is not completely modified by this method of fibre modification due to its amount. The rest of the samples with modified fibres (MF-7% and MF-9%) formed the separate cluster.

The results of HCA based on single linkage method and Euclidean distances are shown in a form of double dendrogram, given in Fig. 3. The grouping of the samples is completely the same as in the case of the application of Ward's method, however this dendrogram is very informative since it shows the similarities of the changes of EC_{50} values during six weeks.

(Please insert Fig. 3 here)

Fig. 3 The double dendrogram as a result of HCA based on single linkage method and Euclidean distances

Based on the colour of the field in double dendrogram, the similarity between two observed objects in the variable space can be revealed. Generally, the most similar EC_{50} values are the values measured in 2nd and 3rd week. The EC_{50} values measured in 1st week significantly differed from the others. Particularly, it can be seen that F-samples mostly differed from the others in 3rd week. These samples had the lowest EC_{50} values in 3rd week. The highest EC_{50} values of the control samples can be observed in 1st week, and the lowest EC_{50} values in 3rd week. The highest difference between control samples and the other samples is expressed in 1st week. After six weeks it can be seen that the antioxidant activity of the samples MF-7% and MF-9% differed mostly from the antioxidant activity of the other samples. The antioxidant activity of the other samples is similar after six weeks.

In the next step of the chemometric analysis, the SRD analysis was carried out. The new graphical result is shown in Fig. 4. The ranking analysis of the samples was based on the reference ranking defined by minimum row values. Therefore, the results indicate that the samples with generally lowest EC_{50} values are F-9%, F-7% and F-11%. These samples are the closest to the reference ranking. The samples MF-7% and MF-11% have the highest distance from the reference ranking. These samples generally have the highest EC_{50} values.

The SRD analysis indicates the groupings of the samples, as well. First group contains the F-samples, the second group are the control samples and NMF-9% sample, the third group contains NMF-7%, NMF-11% and MF-9% samples, while the samples MF-7% and MF-11% belong to the fourth group. The grouping of the samples achieved by the SRD analysis is similar to the groupings obtained by HCA. The similarity refers to grouping of F-

samples and, particularly, control samples. The groupings are not completely the same, since the SRD and HCA methods are essentially different approaches.

(Please insert Fig. 4 here)

Fig. 4 The graphical result of SRD-CRRN analysis of EC_{50} values of the analysed samples (The statistical characteristics of theoretical distribution function are the following: first icosaile (5%), XX1 = 8; first quartile, Q1 = 14; median, Med = 16; last quartile, Q3 = 18; last icosaile (95%), XX19 = 24)

On the basis of the presented chemometric results, considering EC_{50} values, it can be concluded that the control samples and samples with Fibrex® fibres significantly differ from the others and mutually. The results indicate that there is no strict difference between the samples with modified and non-modified fibres.

Conclusion

Regarding the EC_{50} results during six weeks it can be concluded that introduction of NMF and Fibrex® fibres in the formulation of the cookies can improve their antioxidant activity and functional characteristics in the long term effect. However, MF proved to have 50-70% higher antioxidant activity in the four week period after the formulation of the cookies. Therefore, MF should be used for improving antioxidant activity and hydration characteristics of short term bakery products which are intended to be used in the first four weeks. Cookies with Fibrex® fibres had 20% and 40% higher antioxidant activity than cookies with NMF and MF, respectively.

Acknowledgements

The authors acknowledge the financial support of Ministry of Science and Technological Development of the Republic of Serbia.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with Ethics Requirements No human participants and animals were involved.

References

1. Asp, N.-G. (2004). Dietary fibers bio-active carbohydrates for food and feed. Wageningen Academic Publisher, The Netherlands

2. Slavin, J. (2003). Impact of the proposed definition of dietary fiber on nutrient databases. J Food Compos Anal, 16(3), 287-291.

3. Kritchevsky, D. (2001). Dietary fiber in health and disease. In B. V. McCleary & L. Prosky (Eds.), Advanced dietary fiber technology (pp. 149-161). Oxford: Blackwell Science.

4. McKEE, L., & Latner, T. A. (2000). Underutilized sources of dietary fiber: A review. Plant Food Hum Nutr, 55(4), 285-304.

5. DeVries, J. W. (2003). On defining dietary fibre. Proceedings of the Nutrition Society, 62, 37-43

6. Serpen, A., Capuano, E., Fogliano, V., & Gökmen, V. (2007). A new procedure to measure the antioxidant activity of insoluble food components. J Agr Food Chem, 55(19), 7676-7681.

7. Laurikainen, T., Harkonen, H., Autio, K., & Poutanen, K. (1998). Effects of enzymes in fiber-enriched baking. J Sci Food Agr, 76, 239-249.

8. Vural, H., Javidipour, I., & Ozbas, O. O. (2004). Effects of interesterified vegetable oils and sugar beet fiber on the quality of frankfurters. Meat Sci, 67(1), 65-72.

9. Ang, J. F., Crosby, G. A. (2003). A new look at sugar beet fiber. Cereal Foods World, 48(5), 238-243.

10. Köksel, H.; Özboy, O. (1999): Effects of sugar beet fiber on cookie quality. Zuckerind. 124, 542–544

11. Gyura, J., Šereš, Z., Sakač, M., & Mišan, A. (2009). Physico-chemical characteristics of filler additives from sugar beet for application in the production of bread and cookies. Sugar Industry/Zuckerindustrie, 134(9), 593-600.

12. Sakač, M., Gyura, J., Mišan, A., Šereš, Z., Pajin, B., van der Kamp, J. W., ... & Topping, D. L. (2010). Antioxidant properties of cookies supplemented with sugar beet dietary fibre. Dietary Fibre: New Frontiers for Food and Health, 441.

13. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol, 28(1), 25-30.

14. Hęś, M., Dziedzic, K., Górecka, D., Drożdżyńska, A., & Gujska, E. (2014). Effect of boiling in water of barley and buckwheat groats on the antioxidant properties and dietary fiber composition. Plant Food Hum Nutr, *69*(3), 276-282.

15. AOAC International. (2005). Official methods of analysis of AOAC International. AOAC International.

16. Reinefeld, E., & Schneider, F. (1983). Analitische Betriebskontrolle der Zuckerindustrie. Berlin: Verlag Dr. Albert Bartens (A 5.2. pp. 67–68, B 1.5.1. pp. 2–3).

17. Gould, J.M. (1989). Alkaline peroxide treatment of agricultural byproducts. US patent no. 4,806,475

18. Gould, J.M. (1987). Alkaline peroxide treatment of non-woody lignocelluloses. US patent no. 4,649,113

19. Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin, 36, 2090-2097.

20. Brereton, R. G., (2003). Chemometrics: Data analysis for the laboratory and chemical plant, Chichester: John Wiley & Sons Ltd.

21. Miller, J. N., and Miller, J. C. (2010). Statistics and Chemometrics for Analytical Chemistry, 6th edition, Harlow: Pearson Education Limited.

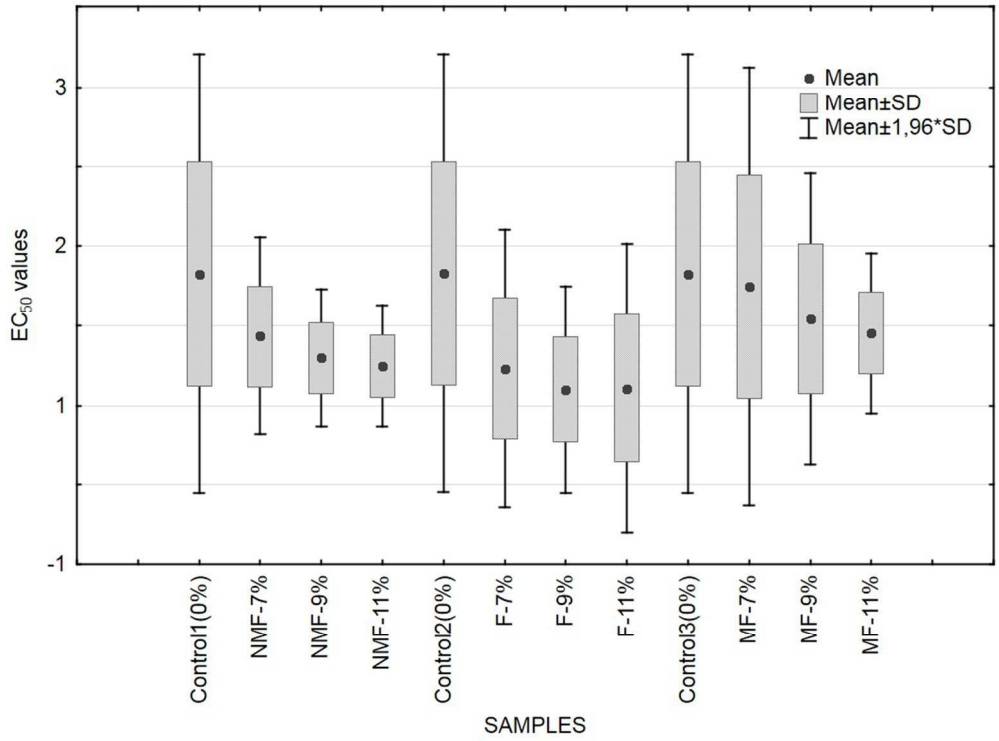
22. Malbaša, R., Jevrić, L. R., Lončar, E. S., Vitas, J., Podunavac-Kuzmanović, S. O., Milanović, S., and Kovačević, S. Z., (2014). Chemometric approach to texture profile analysis of kombucha fermented milk products. J Food Sci Technol, DOI: 10.1007./s1317-014-1648-4

23. Héberger, K., and Kollár-Hunek, K., (2011). Sum of ranking differences for method discrimination and its validation: comparison of ranks with random numbers. J Chemometr, 25, 151-158.

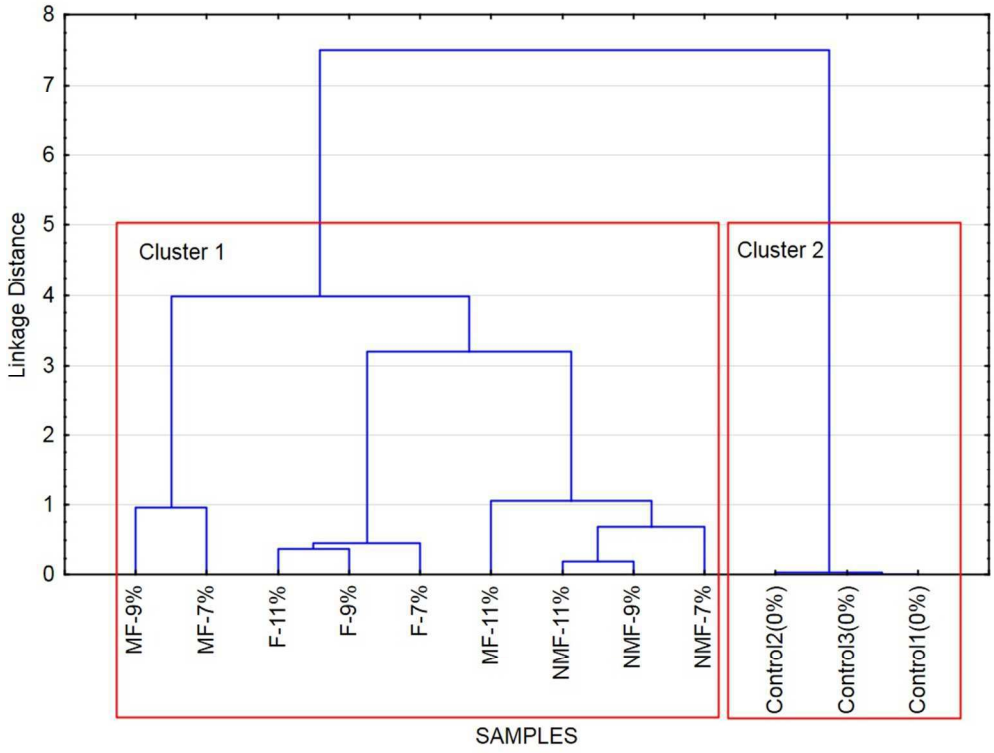
24. Héberger, K., (2010). Sum of ranking differences compares methods or models fairly. Trends in Analytical Chemistry, 29 (1), 101-109.

25. Grigelmo-Miguel, N., & Martín-Belloso, O. (1999). Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. LWT-Food Sci Technol, 32(8), 503-508.

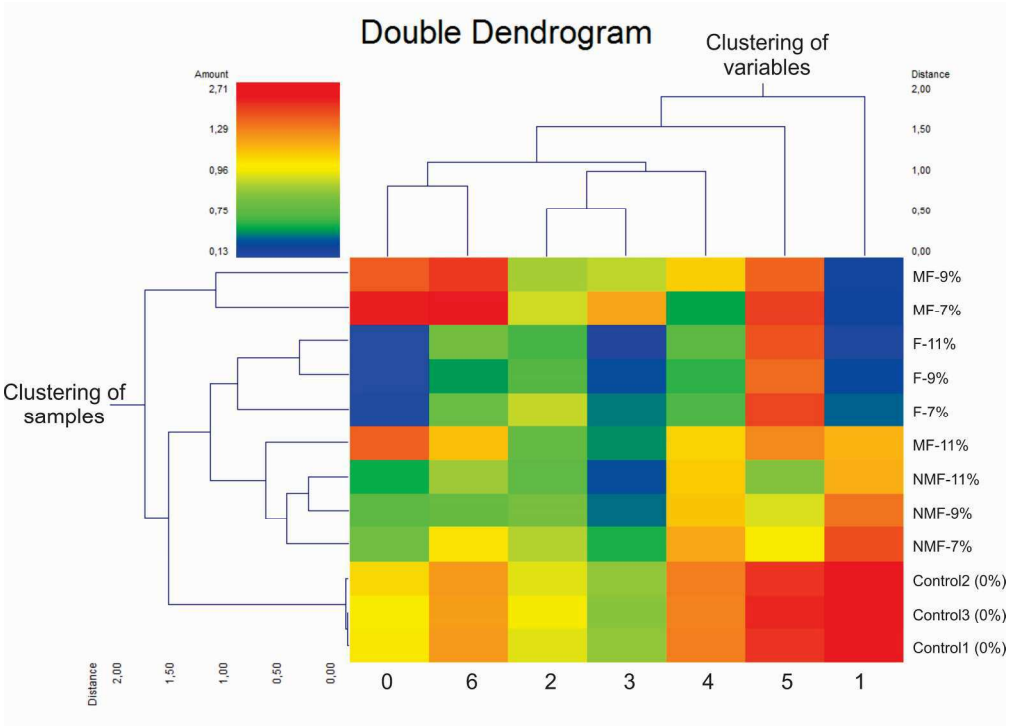
26. Sakamoto, T., & Sakai, T. (1994). Protopectinase-T: a rhamnogalacturonase able to solubilize protopectin from sugar beet. Carbohydrate research, 259(1), 77-91.



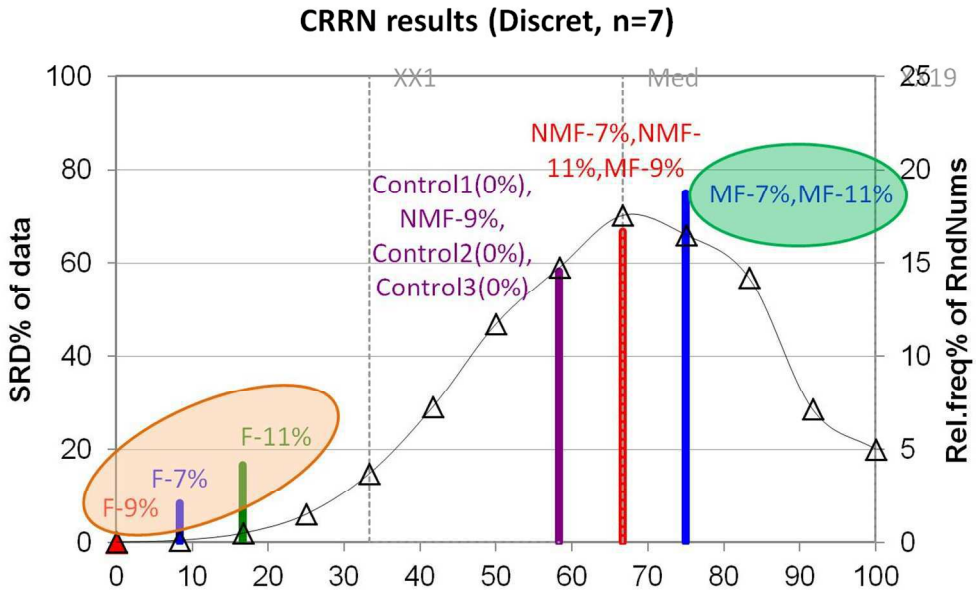
280x211mm (96 x 96 DPI)



280x211mm (96 x 96 DPI)



101x73mm (600 x 600 DPI)



289x173mm (120 x 120 DPI)

Table 1. Basic formula for control cookies (without sugar beet fibre)

Components	%(w/w)
Wheat flour T-500	56.97
Powdered sugar	19.94
Salt	0.31
Vegetable fat	11.97
$NaHCO_3$	0.17
NH_4HCO_3	0.11
H_2O	10.53

Table 2. Physicochemical composition of NMF, Fibrex®, MF

	NMF*	Fibrex®*	MF*
Proteins (%)	12.07	9.36	11.92
Minerals (%)	3.33	4	3.53
Total dietary fibre (%)	75	78.40	74.63
Insoluble dietary fibre (%)	61.11	64.29	57.70
Soluble dietary fibre (%)	13.89	14.11	16.93
Soluble/insoluble fibre ratio	0.23	0.22	0.29

* All values were calculated on a dry matter basis

Table 3. The input data matrix for chemometric analysis (HCA and SRD)

EC_{50} (g/ml)	Non-modified Fibres (NMF)				Fibrex® Fibres (F)				Modified Fibres (MF)			
Week	Control1 (0%)	NMF-7%	NMF-9%	NMF-11%	Control2 (0%)	F-7%	F-9%	F-11%	Control3 (0%)	MF-7%	MF-9%	MF-11%
0	0.9025	0.6990	0.6466	0.5913	0.9253	0.2412	0.1725	0.1267	0.9020	1.9060	1.3310	1.3080
1	2.7123	1.5708	1.1530	1.0293	2.7126	0.4968	0.4309	0.2574	2.7130	0.3690	0.3640	1.0290
2	0.8737	0.7895	0.7358	0.6583	0.8737	0.8449	0.6360	0.6312	0.8740	0.8500	0.7510	0.6810
3	0.7464	0.6119	0.5115	0.4888	0.7464	0.5572	0.4888	0.3393	0.7460	1.0790	0.8360	0.5660
4	1.1425	1.0474	1.0021	0.9712	1.1425	0.6323	0.6156	0.6495	1.1420	0.5860	0.9530	0.9420
5	1.8139	0.9010	0.8511	0.7442	1.8139	1.6461	1.2560	1.5302	1.8140	1.7660	1.2570	1.1310
6	1.0972	0.9165	0.6833	0.7472	1.0972	0.6902	0.5854	0.7144	1.0970	2.1700	1.8090	1.0270